Subcellular fractionation of a complex proteome (cardiac tissue) allows for enrichment of a subset of low abundant proteins. This permits more in depth analysis of the proteome by reducing the complexity. Adult rat cardiac tissue was made ischemic by ligating the left anterior descending coronary artery in vivo for 1 hour with no reperfusion, and the healthy remote area was differentiated from the ischemic tissue by staining with Evans Blue dye after harvesting the heart. A series of differential centrifugation steps produced nuclear, mitochondrial, cytoplasmic, microsomal, and sarcomeric fractions of rat ischemic and remote healthy tissue. The sarcomeric fractions of the remote vs ischemic cardiac tissue were digested with trypsin, and the peptides were labeled with isobaric tags for relative quantitation (iTRAQ). The labeled peptides were then fractionated with an Agilent 3100 OFFGEL fractionator, which separated the peptides in 12 fractions based on their isoelectric point from pH 3-10. The 12 fractions from the OFFGEL were run on a Dionex U-3000 nano LC coupled to a ThermoFinnigan LTQ running in PQD (pulsed Q dissociation) mode to detect the low mass reporter ions of the isobaric tags on the peptides. The peptides were analyzed with MASCOT (MatrixScience), and Scaffold v2.5.2 Q+ with a minimum of two unique peptides and a level of confidence set at 95%. Five-fold more proteins were identified when the labeled digests were fractionated with OFFGEL compared to no fractionation prior to LC-MS/MS. With a one hour ischemic event, we found approximately 11% of the detected proteins in the sarcomeric fraction had changed at least 1.5 fold. Therefore, this in-solution method incorporating sub-proteomic fractionation in conjunction with OFFGEL separation may be an approach for discovery of relative protein changes in cardiac tissue.

3742-Pos

Contractile Function is Altered by Regulation of HO-1 Activity in Single Adult Rat Cardiomyocytes

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Heme oxygenase-1 (HO-1) is a membrane protein upregulated in response to oxidative stress that confers cardioprotection. The therapeutic potential of HO-1 can be assessed by examining cardiomyocytes survival and function using cobalt protoporphyrin (CoPP) or tin protoporphyrin (SnPP) to increase or inhibit HO-1 activity, respectively. Whether altered HO-1 function affects cardiomyocyte contractility, however, is unknown. Thus, we determined the effects of CoPP (n=11), SnPP (n=15), or PBS (control, n=15) on *in vitro* single intact adult cardiomyocyte contraction and relaxation using video microscopy and calcium imaging (IonOptix) at 0.5, 1 and 2Hz. At 0.5Hz there was no difference in the rate or magnitude of shortening between groups, and all cells had similar fractional shortening (FS) normalized to peak calcium (FS/Ca^{2+}). FS/Ca^{2+} was, however, greater at 1Hz and 2Hz in SnPP-treated cells, while CoPP- and PBS-treated cells maintained similar FS/Ca^{2+} relationships across frequencies ($p<0.05$). Intriguingly, while SnPP-treated cells increased contraction at faster pacing, only 53% of those cells contracting at 0.5Hz beat synchronously at 1Hz, compared to 100% of CoPP-treated cells and 80% of PBS-treated cells. This difference became more pronounced at 2Hz, as only 26% of SnPP-treated cells and 60% of PBS treated cells able to follow stimulation, while 100% of CoPP-treated cells continued to beat synchronously. Similarly, although time to 90% relaxation was not different between groups at 0.5Hz, it was significantly faster in CoPP- vs. SnPP-treated cells at 2Hz ($p<0.05$). These results suggest that increasing HO-1 activity via CoPP treatment maintains cell viability under stress, while SnPP- induced HO-1 inhibition reduces survivability at higher pacing frequencies. The mechanisms behind this action are unknown, but may be partially due to HO-1-induced calcium desensitization of the myofilament.
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3743-Pos

Study of O-Glcnacylation of Contractile Proteins in Cardiac Myofibrils by Enzymatic Labelling

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Phosphorylation occurs on serine and threonine residues and plays important roles in the regulation of contractile proteins. In heart failure changes in levels of phosphorylation are reported in a number of cardiac sarcomeric proteins. *O*-linked-*N*-acetylglucosamine (*O*-GlcNAc) modification is another possible posttranslational modification on serine and threonine residues and recent publications reported mapping of *O*-GlcNAc modification sites in some rat contractile proteins, including myosin heavy chain (MHC), actin, cardiac troponin I and myosin light chains (MLC1 and MLC2). *O*-GlcNAc modification on normal donor hearts (49yr F and unknown) and hypertrophic obstructive cardiomyopathy myectomy samples (33yr M and 42yr M) were studied. Cardiac myofibrils were isolated and the *O*-GlcNAc groups labelled using an enzymatic labelling system in the presence of PUGNAc (inhibits *O*-GlcNAc removal enzyme *O*-GlcNAcase) and protease inhibitors. This method allows coupling of an azido modified *N*-acetylgalactosamine (UDP-GalNAz) to *O*-GlcNAc using the mutant enzyme Y289L β1,4-galactosyltransferase (Y289L GalT). The labelled groups were detected by reacting the azide group with an alkyne bearing the tetramethylrhodamine (TAMRA) fluorescent tag for direct imaging following SDS-PAGE. The gel was post-stained with a total protein stain for analysis with densitometry. The labelling process showed no impact on myofibril protein profiles when native and labelled myofibrils were compared. Preliminary results showed that *O*-GlcNAcylation profiles vary between samples with a total of 7 proteins identified. Strong TAMRA signals from α-actinin and MLC1 were observed in all the four myofibrils samples. In three of the samples the proteins actin, tropomyosin (Tm) and myosin binding protein-C (MyBP-C) were positively labelled. MHC and desmin *O*-GlcNAcylation were observed in one of the four subjects. This enzymatic labelling method will be investigated further for possibility of quantification and methods for mapping sites of these modifications with mass spectrometry will be explored.

3744-Pos

Cannabinoid (CB) Receptors are not Involved in A-955840 Induced Negative Inotropic Effects in Isolated Cardiac Myocytes

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A-955840, a selective CB_2 agonist, has been shown to elicit concentration-dependent decreases in cardiac contractility in the anesthetized dog (decreased maximal velocity of left ventricular pressure development [LV dP/dt max]). However, it is unknown whether this represents a direct effect or a response dependent on other factors (such as autonomic tone and neurohumoral factors) present in vivo. This study examined if A-955840 had a direct effect on contractility of isolated cardiac myocytes, and if so to determine the potential involvement of CB_1 and CB_2 receptors. Contractility was assessed in vitro using percent changes in maximal shortening velocity of sarcomeres (dL/dt max) and fractional shortening of sarcomere length (FS) in rabbit left ventricular myocytes. A-955840 reduced dL/dt max and FS in a reversible and concentration-dependent manner with an IC_{50} of 11.4 µg/mL (based on dL/dt max) which is similar to the IC_{50} value of 5.5 µg/mL based on the effects of A-955840 on LV dP/dt max in anesthetized dogs. A-955840 (4.0 µg/mL) reduced myocyte contractility (%FS) to a similar extent in the absence and presence of a CB_2 antagonist, SR-2 (24.0 ± 3.4 vs. 23.1 ± 3.0 %, n=5) or a CB_1 antagonist, rimonabant (18.8 ± 2.3 vs. 19.8 ± 2.7 %, n=5). A-955840 (4.0 µg/mL) also reduced L-type calcium current of rabbit ventricular myocytes (1.05 ± 0.11 vs. 0.70 ± 0.12 nA, n=5, $P<0.01$). These results suggest that A-955840 exerts direct negative inotropic effects on isolated rabbit ventricular myocytes, which is mediated by neither CB_1 nor CB_2 receptors, and consistent with off-target negative inotropy mediated by inhibition of the cardiac L-type calcium current.

3745-Pos

Effects of Development of Compensatory Hypertrophy on Force Frequency and Beta-Adrenergic Responses

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Background: The progression to heart failure involves two steps: compensatory hypertrophy, a process where contractile function is enhanced and myocyte size is enlarged, is followed by decompensatory hypertrophy and myocardial weakening. In our current study we aim to understand the temporal resolution of the development of compensatory hypertrophy through the analysis of functional and molecular changes. At present, we are assessing the impact of these changes on contractile modulators through analysis of effects on frequency and beta-adrenergic regulation.
Methods: Trabeculae are excised from the right ventricular free wall of New Zealand White rabbits. Thin linear muscle preparations are then suspended in culture media at high preload and stimulated to contract for up to 24 hours at 1 Hz. At 0-2, 6-8, 12, 18, or 24 hours the effects on either frequency-dependent or beta-adrenergic regulation are measured. Data is analyzed in real-time through customized Labview software.
Results: We have shown that during the progression of compensatory hypertrophy the beta-adrenergic response shifts to the left indicating a higher sensitivity with increasing culture time. Changes in frequency from 1 to 4 Hz of trabeculae cultured from 0-24 hours lead to an increase in developed force of 37% at 0-2 hours (n=5), 53% at 6-8 hours (n=7), 20% at 12 hours (n=6), 30% at 18 hours (n=6) and a decrease of 69% at 24 hours (n=4). This suggests that the